

Intro

Amino acid analysis as a technique was first developed by Stein, Moore and coworkers in the 1950s, and, in 1972 they were awarded the Nobel Prize in chemistry for their contribution to the understanding of protein structure. Since then the technique has been refined and improved in speed and sensitivity by the use of automation and high performance chromatography.

There are four steps in amino acid analysis:

- 1) Hydrolysis (6N HCl, 110°C for 20-24 hours)
- 2) Derivatization of the amino acids for detection
- 3) Chromatographic separation of derivatized amino acids
- 4) Data interpretation and calculations

The Biochrom 30 uses the classical amino acid analysis methodology which involves ionexchange chromatography and postcolumn continuous reaction with ninhydrin. The potscolumn ninhydrin–amino acid derivative eluted from column, known as Ruhemann's purple, is monitored at 570 and 440 nm (proline). The resultant chromatogram gives the identity, ratio and amount of the amino acids present in the given sample. The detection limit of this method is ~10 pmol (50 pmol for proline). This method is tolerant with samples which are contaminated with salts, urea, and small amounts of detergents.



Nynhidrin derivatization reaction